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MAIN TEXT

Clinical relevance of cell-free DNA during venovenous extracorporeal membrane oxygenation

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Abstract

Background: Thrombosis remains a critical complication during venovenous extracorporeal membrane oxygenation (VV ECMO). The involvement of neutrophil extracellular traps (NETs) in thrombogenesis has to be discussed. The aim was to verify NETs in the form of cell-free DNA (cfDNA) in the plasma of patients during ECMO.

Methods: A fluorescent DNA-binding dye (QuantifFluor®, Promega) was used to detect cell-free DNA in plasma samples. cfDNA concentrations from volunteers ($n = 21$) and patients ($n = 9$) were compared and correlated with clinical/technical data before/during support, ECMO end and time of a system exchange.

Results: Before ECMO, patients with a median (IQR) age of 59 (51/63) years, SOFA score of 11 (10/15), and ECMO run time of 9.0 (7.0/19.5) days presented significantly higher levels of cfDNA compared to volunteers (6.4 (5.8/7.9) ng/μL vs. 5.9 (5.4/6.3) ng/μL; $p = 0.044$). Within 2 days after ECMO start, cfDNA, inflammatory, and hemolysis parameters remained unchanged, while platelets decreased ($p = 0.005$). After ECMO removal at the end of therapy, cfDNA, inflammation, and coagulation data (except antithrombin III) remained unchanged. The renewal of a system resulted in known alterations in fibrinogen, D-dimers, and platelets, while cfDNA remained unchanged.

Conclusion: Detection of cfDNA in plasma of ECMO patients was not an indicator of acute and circuit-induced thrombogenesis.

KEYWORDS

blood, cell-free DNA, coagulation, ECMO, inflammation, neutrophil extracellular traps

1 | INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) is a life-saving technology for patients with cardiovascular and/or respiratory failure.[1] Despite improvement of ECMO technology, experience and anticoagulation, the invasiveness of ECMO creates a high risk of potentially fatal complications (bleedings, thrombosis) that affect morbidity and mortality.[2–5] Non-physiological shear forces and the contact of the patient's blood with artificial surfaces

within the extracorporeal circuit are associated with an immediate activation of both the inflammatory response and the coagulation cascade.[1,6–8] As a result, 30%–50% of all ECMO patients develop thrombotic complications.[4,9] When occurring within the membrane oxygenator, these complications are still unpredictable and may lead to equipment malfunction and circuit loss, necessitating an emergency high-risk system replacement.[4,10] The underlying mechanisms are unclear but include the activation of platelets, neutrophils, and the coagulation system.[1,7,11]

Neutrophils and their specific antimicrobial mechanism—the expulsion of their DNA and granular proteins forming an extracellular matrix, termed neutrophil extracellular traps (NETs, NETosis)[12]—have gained much interest in the context of vascular and material-associated thrombosis.[13–18] Cell-free DNA (cfDNA) is thought to derive primarily from dead cells of the hematopoietic lineage; however, cfDNA is also a major structural component of NETs and triggers the intrinsic pathway of blood coagulation.[13,19] cfDNA as well as components of NETs were not only found in human thrombi[20–22] but circulate also in human blood[23,24] as a possible biomarker of NETosis in thrombotic pathologies. Matros et al. showed elevated levels of cfDNA in a large case–control study of VTE (venous thromboembolism) patients compared to healthy controls and was associated with an increase in the thrombotic risk.[23] Valles et al. demonstrated high NET levels in patients with ischemic stroke that correlated with stroke severity.[24] In the case of ECMO, adherent leukocytes on gas exchange membranes from ECMO oxygenators proved the involvement of inflammatory reactions in ECMO-induced thrombus formation.[15,25–28] Furthermore, cfDNA and other NET components were also detected in plasma of venoarterial ECMO patients and found in thrombi within ECMO circuits.[29]

This study evaluates the hypothesis that cfDNA is detectable in blood plasma from patients that required VV ECMO. System exchanges as well as weaning from ECMO were used as a scenario of membrane clotting with alterations of cfDNA levels.

2 | MATERIALS AND METHODS

2.1 | Patients, settings, and sample preparation

This study was a prospective observational study and approved by the Ethics Committee of University Medical Hospital Regensburg (vote no 16-101-0322). Written informed consent was obtained from nine patients (or the legal representatives) who required VV ECMO due to acute lung failure and from 21 healthy volunteers. Blood samples were collected in EDTA tubes. From December 2017 to May 2019, blood samples were collected before, 2, 6, 24, and 48 h and every 5 days after ECMO implantation, after ECMO end and in selected cases before and after a system exchange. The outbreak of the Covid-19 pandemic prevented the inclusion of more patients and an earlier evaluation of the data. ECMO exchange reasons were defined according to Lubnow et al.[4] (Table S1).

Ficoll-Paque PREMIUM (GE Healthcare Europe, Freiburg, Germany) solution (3 mL) was filled into a centrifuge tube and carefully layered by 1.4 mL of anticoagulated blood. After centrifugation (400 *g*, 30 min, room temperature, RT), the upper layer was carefully removed and frozen at −80°C until further processing. Samples were blinded before further processing.

2.2 | Detection of cfDNA in plasma samples

The QuantiFlour® dsDNA System (Promega, Madison WI, USA) was used to detect and quantify double-stranded DNA (dsDNA) in plasma samples from patients and healthy volunteers.[30] Thawed plasma samples were centrifuged (20 817 *g*, 30 min, 4°C) and soluble dsDNA (= cfDNA) was analyzed in the supernatant. According to manufacturer's instructions, 2 µL of 1:5 pre-diluted plasma was mixed with 200 µL of QuantiFluor® dsDNA Dye working solution and fluorescence ($504\,nm_{Ex}/531\,nm_{Em}$) was measured using the Quantus™ Fluorometer (Promega). Lambda DNA was used as a standard (final mass, 0.05 to 200 ng). cfDNA (ng) of the samples were determined from the standard curve (considering the used sample volume) using weighted least squares linear regression (WLSR)[31] (Figure S1).

$$\text{WLSR: m(cfDNA) [ng]} = (\text{fluorescence intensity [RFU]} - 6.7)/62$$

Stimulated neutrophils were used as a positive control for the detection of cfDNA. After in vitro stimulation of isolated neutrophils with PMA (phorbol-12-myristate-13-actate), released cfDNA from different neutrophil concentrations served as controls. Neutrophils were isolated from 30 mL Lithium-heparin-anticoagulated blood from a healthy volunteer. Leukospin medium, Lymphospin medium (both from pluriSelect Life Science, Leipzig, Germany) and anticoagulated blood (each, 3 mL) was layered in 10 centrifugation tubes (Falcon tube, Corning, NY, USA) and centrifuged (800 *g*, without brake, 20 min, RT). The neutrophil pellet was separated into five new centrifugation tubes, each adjusted to 10 mL with cold phosphate buffered saline plus bovine serum albumin (PBS/BSA 0.5%), and centrifuged (250 *g*, 10 min, 4°C). The supernatant was discarded and the cell pellets were each carefully resuspended in a total of 5 mL

of RPMI-medium including 3% fetal bovine serum (FBS). Cell count was measured with CASY-TTC (OMNI Life Science, Bremen, Germany). Cells were twofold diluted in RPMI-medium/FBS. Aliquots (750 µL) of the diluted cell suspensions were seeded into 12 well-plates (Corning) and stimulated with equal volumes of PMA (200 nM, diluted in RPMI/3% FBS) or RPMI/3% FBS (non-stimulated control) (3 h, incubator at 37°C). After discarding the supernatant, adhered cells were extensively washed with cold PBS (twice, each, 625 µL). Flushing solutions were pooled and centrifuged (450 *g*, 10 min, 4°C). The supernatant was centrifuged again (20 817 *g*, 30 min, 4°C) and frozen (−80°C) until determination of the concentration of cfDNA as described above.

2.3 | Data collection and statistical analysis

Patient data were collected prospectively (Regensburg ECMO database) and pseudonymized. Parameters of particular interest were inflammation related (C-reactive protein, CRP; leukocyte count; tumor necrosis factor, TNF; interleukin-6, IL-6), hemolysis related (lactate dehydrogenase, LDH; plasma free hemoglobin, fHb) and coagulation related (fibrinogen, FG; D-dimers, DD; platelet counts; antithrombin III, ATIII; activated partial thromboplastin time, aPTT; International Normalized Ratio, INR).

Statistical analysis included the kinetics of selected laboratory parameters within the first 2 days after ECMO implantation, before and after ECMO end and before and after a system exchange. Statistical analysis was done using SigmaStat 3.5 (SYSTAT Software, San Jose, CA, USA). Continuous variables were shown as median (interquartile range, IQR), categorical variables are expressed as frequencies (percentage). Two-way analysis of variance (ANOVA) was used to compare the timeline of specific parameters. If there is a statistical significant difference, the parameters at indicated time points were compared using one-way ANOVA. The Chi-square test was used if nominal distributed parameters were to be tested for correlation. A p-value <0.05 was considered the threshold of statistical significance.

3 | RESULTS

3.1 | Study population and ECMO run data

The group of healthy volunteers consisted of 13 men and eight women with a median (IQR) age of 24 (23/25) years.

The median age of seven male and two female patients was 59 (51–63) years with elevated inflammatory data. Baseline characteristics are summarized in Table 1. VV ECMO was implanted due to aspiration (n=1), bacterial (n=2), and viral (n=5) pneumonia, and acute lung failure post surgery (n=1). The median SOFA score was 11 (10/15). One patient required renal replacement therapy. Patients were anticoagulated with argatroban (n=4) and heparin (n=5).

TABLE 1 Characteristics and basal laboratory data.

Number (n)	9
Age (years)	59 (51–63)
Male gender, n (%)	7 (78)
BMI (kg/m^2)	26.3 (24.1/30.8)
Ventilation (days)	1 (0–12)
SOFA score	11 (10/15)
LIS	3.3 (3.3/3.7)
ECMO indication, n (%)	
Pulmonary ALF	8 (89)
Extrapulmonary ALF	1 (11)
Primary anticoagulation (n)	4× Arg/5× Hep
White blood cells (×10^9/L)	13.7 (10.0/14.3)
C-reactive protein (mg/L)	223 (135/288)
Interleukin-6 (pg/mL)	609 (243/8069)
Interleukin-8 (pg/mL)	283 (49/824)
Tumor necrosis factor (pg/mL)	32 (16/64)
INR	1.1 (1.0/1.3)
aPTT (s)	42 (34/44)
Platelets (×10^9/L)	211 (139/250)
D-dimers (mg/L)	6 (4/10)
Fibrinogen (g/L)	475 (382/581)
Antithrombin III (%)	56 (47/74)
Free hemoglobin (mg/L)	31 (26/60)
LDH (U/L)	320 (202/519)

Note: Data are shown as median (interquartile range); except for male gender; ECMO indication (n, %); pulmonary ALF, 2× bacterial, 5× viral, 1× aspiration pneumonia; extrapulmonary ALF, ALF post surgery.

Abbreviations: ALF, acute lung failure; aPTT, activated partial thromboplastin time; ARF, acute renal failure; BMI, body mass index; INR, international normalized ratio; LDH, lactate dehydrogenase; LIS, Murray lung injury score; RRT, renal replacement therapy before ECMO; SOFA, Sequential Organ Failure Assessment.

There was no prevalence for a specific ECMO system. Eight patients were supported with single-lumen cannulas. One double-lumen cannula was implanted (Table S2). The median total ECMO time was 9.0 (7.0/19.5) days. Three patients required more than one system (n=14, Table 2). Exchange reasons based on the development of thrombotic deposits within the ECMO system verified as circuit-induced coagulation disorder (COD, n=8 [57%]), pump head thrombosis (PHT, n=4 [28%]), worsening of the gas transfer of the oxygenator (WGT, n=1 [7%]), acute oxygenator thrombosis (AOT, n=1 [7%]).[4] Seven patients were successfully weaned

TABLE 2 ECMO run data, outcome, and transfusion requirement.

Parameter	Value
Number (*n*)	9
Total ECMO time (days)	9.0 (7.0/19.5)
Cumulative ECMO time (days)	285
Run time 1st MO (days)	9.0 (7.0/15.0)
Patients with exchanged systems, *n* (%)	3 (33)
Number of exchanges of the three patients with a system exchange, *n* (sum)	1, 2, 11 (14)
RRT during ECMO, *n* (%)	4 (44)
Survival to hospital discharge, *n* (%)	7 (78)
Total transfusion	
RBC/day	0.44 (0.35/0.50)
FFP/day	0.00 (0.00/0.00)
PC/day	0.00 (0.00/0.06)

Note: Data are shown as median (interquartile range); except for patients with exchanged systems, renal replacement therapy before and during ECMO support (RRT), survival to hospital discharge. Run time 1st MO identified the time from ECMO start to the end of the 1st membrane oxygenator (MO) including the exchanged MOs as well as cases with only one MO.

Abbreviations: FFP, fresh frozen plasma (1 FFP contains 230 mL plasma); PC, platelet concentrate (1 PC contains 250 mL and 2–4 × 10^{11} platelets); RBC, red blood cells.

and discharged from hospital. One patient died on ECMO (cerebral bleeding) and one patient died 8 days after weaning (septic circulatory failure). Patients mainly received RBCs (red blood cell concentrates), while only few FFPs (fresh frozen plasma) and PCs (platelet concentrates) were transfused (Table 2).

3.2 | Identification and quantification of cfDNA using a fluorescent DNA-binding dye

cfDNA was significantly higher in the supernatant of stimulated neutrophils compared with non-stimulated neutrophils (Figure S2).

3.3 | Quantification of cfDNA in plasma from healthy volunteers and critically ill patients before ECMO

Plasma samples from healthy volunteers contained significantly less cfDNA compared to those from critically ill patients before ECMO (volunteers vs. patients, 5.86 (5.35/6.28) ng/μL vs. 6.43 (5.81/7.90) ng/μL, $p = 0.044$) (Figure 1).

Bacterial pneumonia entailed no elevated cfDNA levels ($n = 2$, mean cfDNA, 6.64 ± 1.92 ng/μL) compared to patients with viral pneumonia ($n = 5$, mean cfDNA, 7.29 ± 1.42 ng/μL; $p = 0.634$). Two patients died on ECMO or within 8 days after weaning. There was no correlation of initial cfDNA concentrations and survival (cfDNA before ECMO: survivors, (6.41 (5.28/7.64) ng/μL; non-survivors, 8.72 (7.80/9.64) ng/μL, $p = 0.111$).

3.4 | Kinetics of cfDNA in the bloodstream of patients after ECMO start

Within the first 2 days after ECMO start, blood sampling and clinical data were completed for all patients. The concentration of cfDNA and other documented inflammatory parameters remained unchanged over time (Figure 2). Selected hemolysis and coagulation parameters did not change within 2 days after ECMO start, except for a significant decrease of platelet counts ($p = 0.005$) and increasing INR ($p = 0.028$) and aPTT ($p = 0.049$) values (Figure 3). Blood flow remained unchanged within the first 2 days after ECMO start (Figure S3A). Differences in anticoagulation had no influence on the analyzed parameters [argatroban vs. heparin on day 2 after ECMO start; cfDNA, 6.15 (4.20/7.63) ng/μL vs. 6.65 (5.77/7.25) ng/μL, $p = 0.905$; platelets, 146 (99/166) /nL vs. 98 (52/187) /nL, $p = 0.413$; INR, 1.4 (1.3/1.5) vs. 1.2 (1.1/1.5), $p = 0.413$; aPTT, 48 (45/98) vs. 57 (80/60) s, $p = 0.286$].

3.5 | Presence of cfDNA in the bloodstream before and after termination of ECMO

Weaning from the ECMO system is equivalent to removing artificial surfaces from the bloodstream. Five

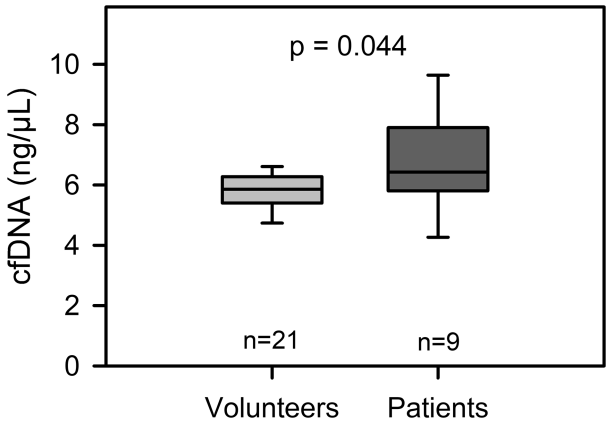

FIGURE 1 Determination of cfDNA in blood samples from healthy volunteers ($n = 21$) and critically ill patients before ECMO ($n = 9$). Boxplots include median and IQR with significantly higher levels for the patients (one-way ANOVA).

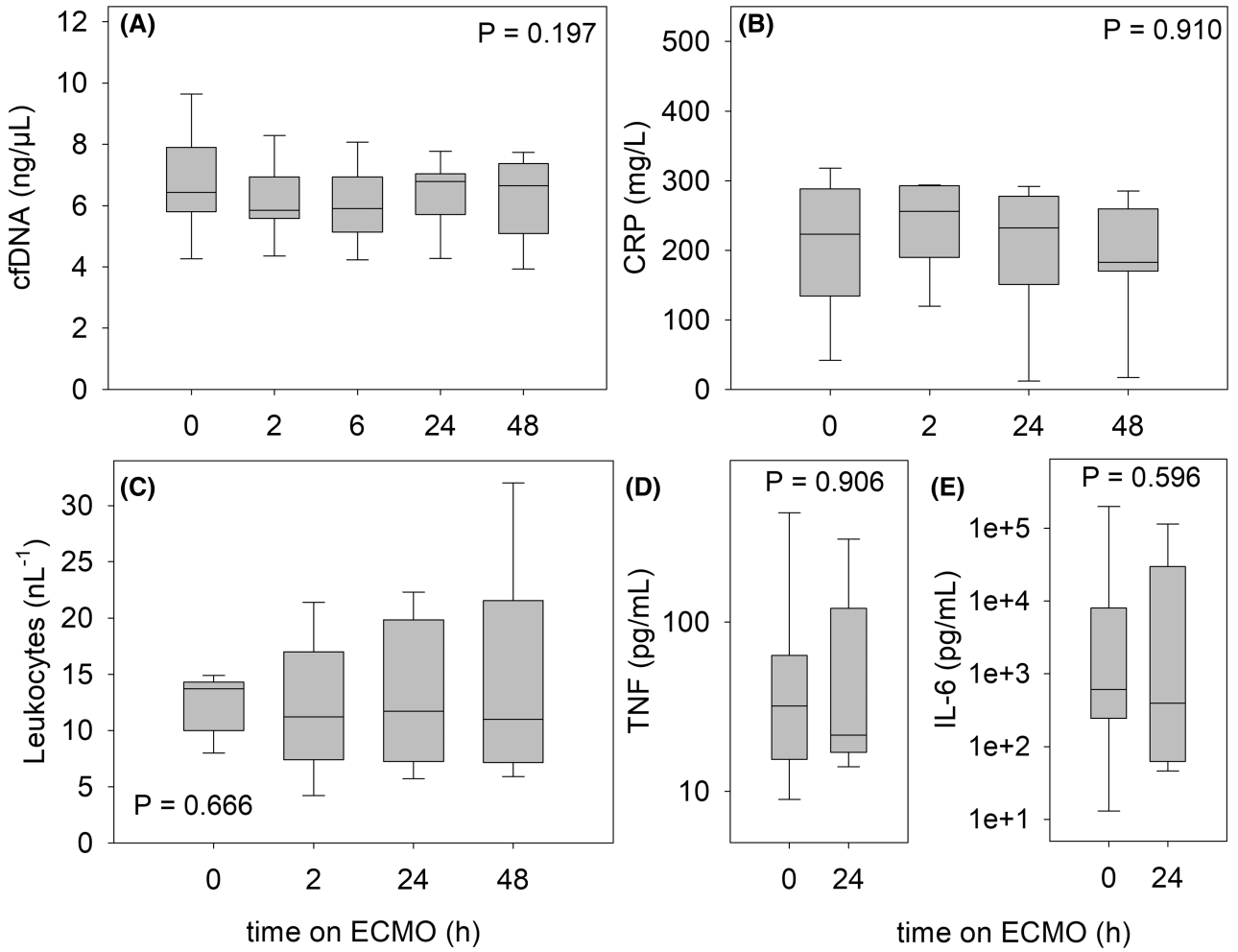

FIGURE 2 Kinetics of cfDNA and other inflammatory parameters within 48 h after ECMO start ($n = 9$). Concentrations of (A) cfDNA, cell-free DNA; (B) CRP, C-reactive protein; (D) TNF, tumor necrosis factor, and (E) IL-6, interleukin-6, and (C) leukocyte counts remained unchanged. Boxplots include median and IQRs. Statistics used two-way ANOVA.

patients allowed the comparison of inflammation, coagulation, and hemolysis data before (2 to 5 days) and after (1 to 2 days) termination of ECMO therapy. While there were no differences in cfDNA, CRP, IL-6, leukocyte count, fibrinogen, D-dimers, platelet counts, INR, aPTT, LDH, and fHb, only ATIII presented significantly higher values before and after end of ECMO compared to pre ECMO (Figure 4). Blood flow decreased from day 2 before to the day of ECMO end ($p < 0.001$, Figure S3B). The levels of cfDNA of the two non-survivors (5.7 and 9.9 ng/µL) remained in the same range compared to the other patients.

3.6 | Presence of cfDNA in the bloodstream at the time of a system exchange

The need for a system exchange always arises from the activation of coagulation with a deterioration in gas transfer performance and the formation of clots within the system.[4] Three patients (33%) required a system exchange. Of the total amount of system exchanges ($n = 14$, Table 2) blood samples before, the day of, and after exchange were only available for eight cases from one patient with complete data sets. Systems were exchanged due to COD ($n = 4$), PHT ($n = 2$), WGT ($n = 1$), and AOT ($n = 1$). Blood sampling for cfDNA detection was inhomogeneous before and after exchange. The first samples before (1 to 7 days) and after (1 to 4 days) exchange were compared to the day of exchange (Figure 5). The levels of cfDNA showed a decreasing trend before and an increasing trend after exchange ($p = 0.054$). Fibrinogen, D-dimers, and platelets worsened before and improved after exchange (Figure 5E–G). In contrast, leukocytes and CRP, INR, aPTT, ATIII, LDH, and free hemoglobin remained unchanged. Blood flow remained unchanged during the exchange procedure (Figure S3C). Excluding cases with PHT ($n = 2$), cfDNA remained unchanged during the exchange procedure ($p = 0.107$; Figure S4).

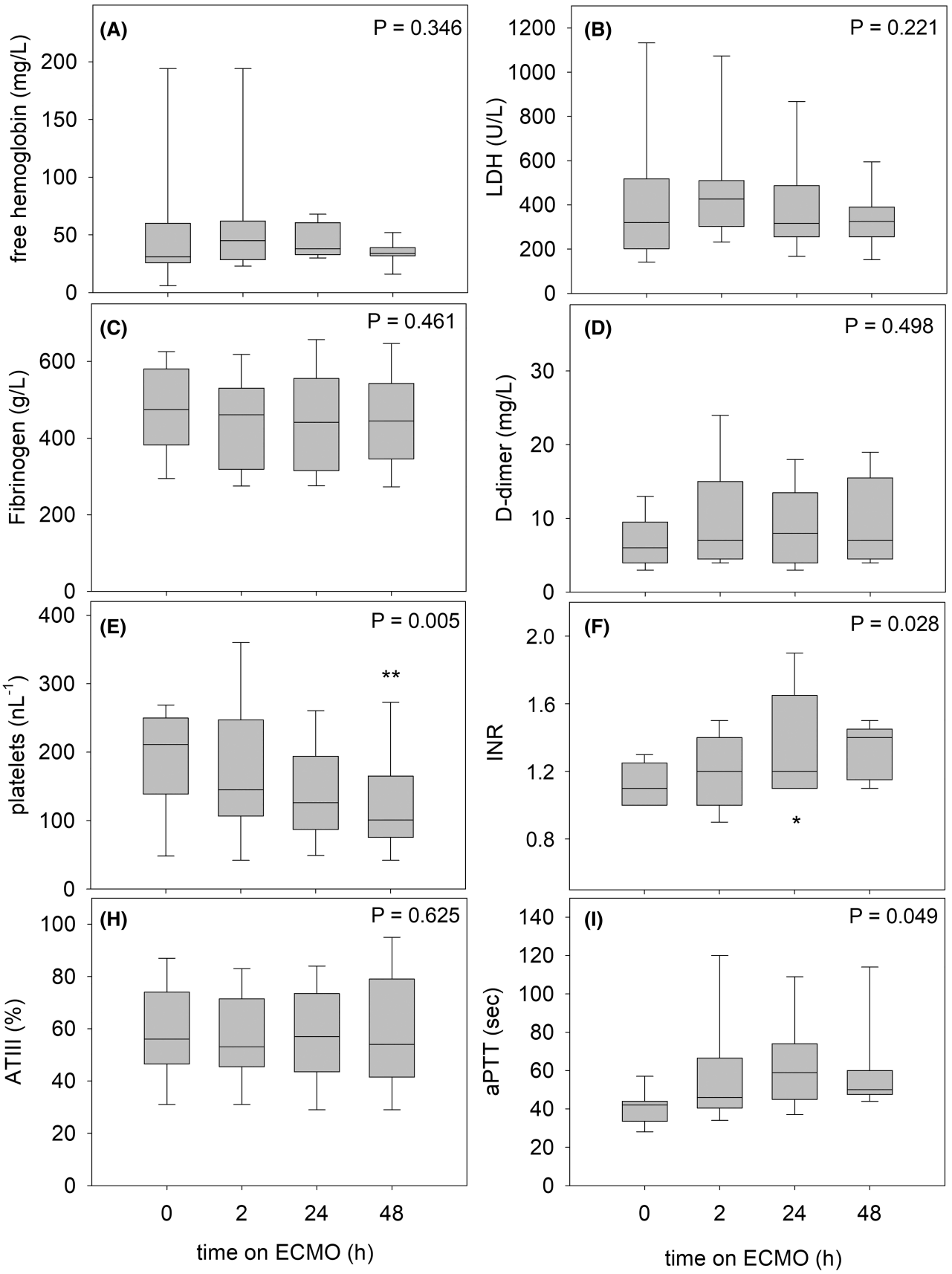

FIGURE 3 Kinetics of hemolysis and coagulation parameters within 48 h after ECMO start ($n = 9$). Free hemoglobin (A), LDH (B), fibrinogen (C), D-dimers (D), and ATIII (G) remained unchanged, while platelets (E), INR (F), and aPTT (H) changed over time. Boxplots include median and IQR. *$p < 0.05$; **$p < 0.01$, Two-way ANOVA, comparing respective time point with pre-ECMO (0 h).

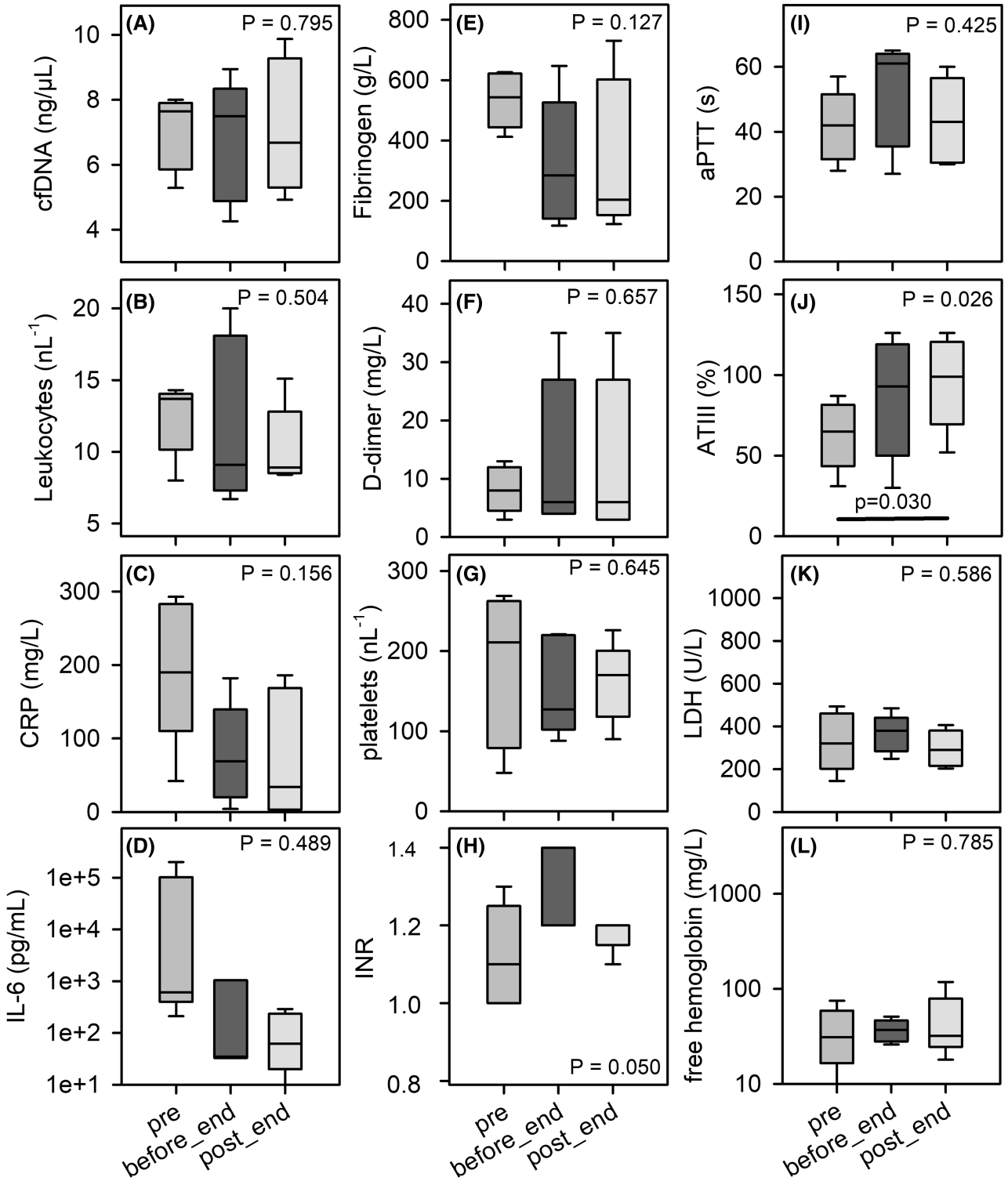

FIGURE 4 cfDNA, other inflammation, coagulation, and hemolysis data before ECMO (pre), before (before_end), and after (post_end) termination of ECMO ($n = 5$). cfDNA (A), Leukocytes (B), C-reactive protein (C), interleukin-6 (D), fibrinogen (E), D-dimer (F), platelets (G), INR (H), aPTT (I), LDH (K), and free hemoglobin (L) remained unchanged, while ATIII (J) was significantly different comparing the three time points. Boxplots include median and IQR. Statistics used two-way ANOVA.

4 | DISCUSSION

The present study presented cfDNA in the blood from patients that required VV ECMO. cfDNA was significantly elevated in the critically ill patients compared to healthy controls. Neither the implantation of an ECMO system nor the exchange of a system and the termination of ECMO therapy altered cfDND levels in blood samples.

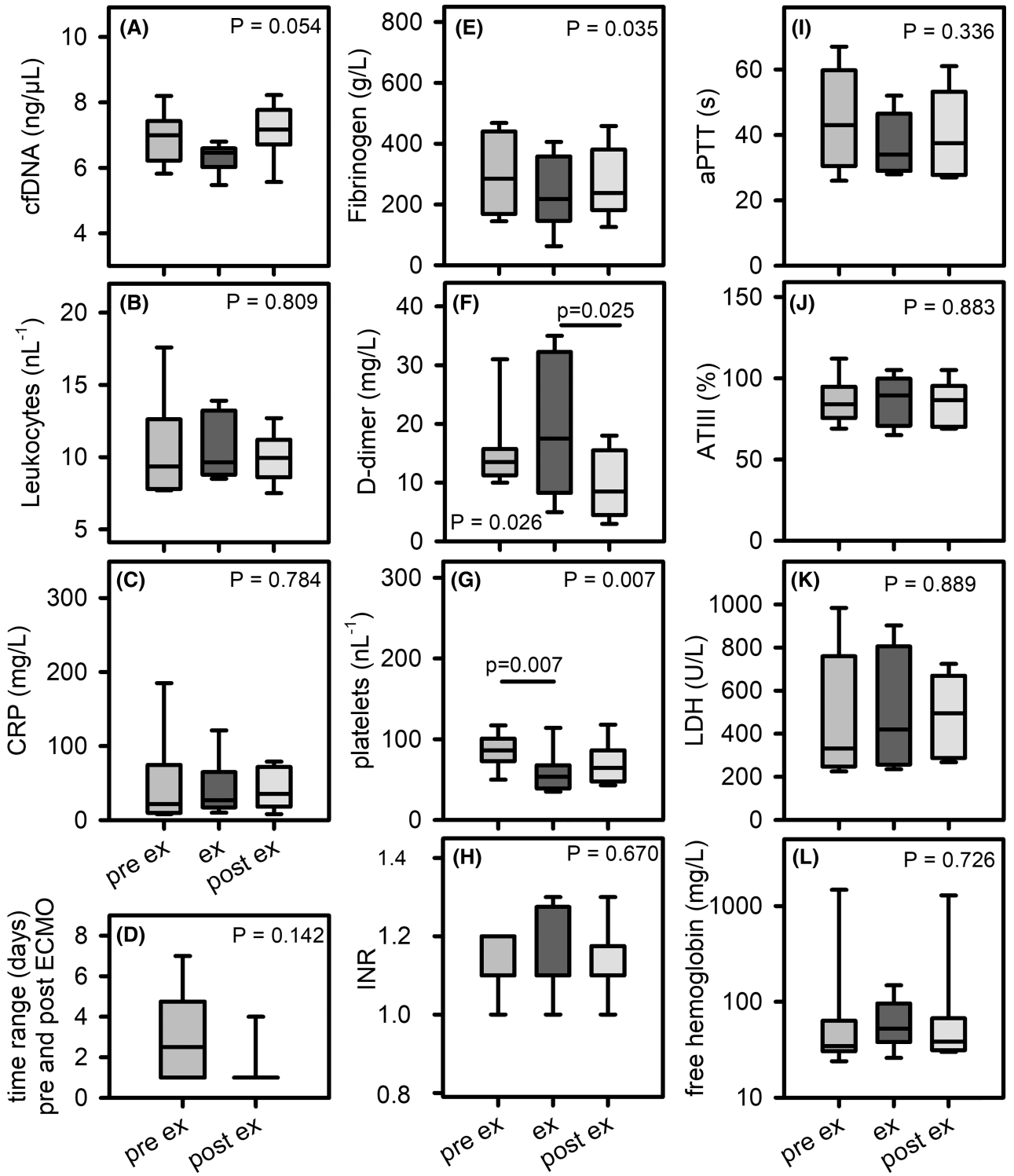

FIGURE 5 cfDNA, other inflammation, coagulation, and hemolysis data before (pre_ex), day of (ex) and after (post_ex) exchange of an ECMO system ($n = 8$). cfDNA (A), leukocytes (B), C-reactive protein (C), INR (H), aPTT (I), ATIII (J), LDH (K) and free hemoglobin (L) remained unchanged, while coagulation parameters changed including fibrinogen (E), D-dimer (F), and platelets (G). (D) The time range of sampling for cfDNA measurement pre and post exchange. Boxplots include median and IQR. Statistics used two-way ANOVA.

Before ECMO therapy, all blood samples of our ARDS patients presented significantly elevated levels of cfDNA compared to healthy controls. The control group was not age and sex matched to the patients. Seventy-eight percent of our patients required VV ECMO due to viral and bacterial pneumonia. Although NETs are used to trap bacteria, cfDNA levels of our patients with bacterial pneumonia before ECMO start remained in the

same range as in patients with viral and other pneumonias. However, the results are questionable due to the low sample size and non-specificity of the test system. Nevertheless, Lefrancais et al. confirmed the presence of higher plasma levels of NETs in ARDS subjects with infectious etiology that correlated with the worst clinical outcomes.[32] In contrast, in the present study, cfDNA levels of patients that died on ECMO or shortly after weaning were not elevated compared to survivors. However, the sample size was too small for valuable statistics. In addition, patients with moderate or severe pneumonia-related ARDS exhibited significantly higher bronchoalveolar lavage fluid NET concentrations than non-ARDS controls, whereas the difference of serum concentrations did not reach statistical significance ($p = 0.072$).[33] However, both studies used a NET-ELISA technique by measuring citrullinated histone H3 (citH3) or myeloperoxidase-DNA complexes to quantify NET levels. A recently published ECMO study approved higher plasma levels of dsDNA, citH3, and nucleosomes in ECMO patients compared to healthy controls.[29] However, the authors only analyzed blood from ICU patients 6 h after establishing venoarterial ECMO. The initial NET levels of these patients before ECMO implantation are missing. Therefore, the influence of the critical illness of the patients on the NET level was neglected. The authors also demonstrated the release of cfDNA in healthy sheeps that were treated with VV or VA ECMO. They observed a 30% significant increase after 6 h with subsequent high variations up to day 5 on ECMO.[29] In the present study, the implantation of an ECMO circuit had no additional effect on the levels of cfDNA of critically ill patients.

As mentioned above, an isolated peak level of dsDNA and citH3 protein in the blood was shown in healthy sheep within 6 h after ECMO implantation.[29] It was striking that the concentration of both NET markers reached the initial value after 24 h and did not change over the next 3 days. It is questionable whether this peak is sufficient to establish a correlation to the involvement of NETs in thrombus formation within the oxygenator.[29] In the present study, there was no alteration in cfDNA and other inflammatory parameters. In contrast, Zhang et al. demonstrated significantly higher leukocyte counts, in particular neutrophils, within 1 day on ECMO.[29] As a limitation, it must be noted that no distinction was made between VA and VV ECMO. However, both ECMO modes induced an increase of neutrophils within 1 day to the same extent.[34] Contact of blood and artificial surfaces also activates the coagulation cascade. In the present study, only platelet counts significantly decreased within 48 h on ECMO. This was in agreement with Malfertheiner et al.[35] The authors demonstrated significant differences within 5 days after ECMO implantation—platelet counts, factor XIII, fibrinogen, CRP, PMN-elastase, IL-6, IL-8 decreased, while F1.2, TAT complex, D-dimers, ATIII increased and hemolysis parameters remained unchanged.[35] Most alterations of kinetic data only became significant after 3 to 5 days, so the timeframe used in the present study may have been too short to confirm the differences. The drop of platelets after implantation of a mechanical circulatory support (MCS) is a common phenomenon that is reversible after explantation.[36] In the present study, no recovery of the examined parameters was determined after explantation of the ECMO system. The reason for this could be the small number of patients and the high interindividual variability.

The release of NETs into blood represents an important mechanism in the development of acute thrombi.[20,37] There is only one study that suggested that the presence of NETs (cfDNA and citH3) in the blood of ECMO patients is a biomarker for thrombus formation during ECMO support.[29] Therefore, alterations in the levels of cfDNA should correlate with the development of a circuit-induced coagulation disorder that necessitates the exchange of the system. However, at the time of a system exchange, the levels of cfDNA remained unchanged. Therefore, the detection of cfDNA is not a biomarker for acute coagulation activation within the ECMO system. Instead, coagulation disorder was proven as described by Lubnow et al.[4] However, the meaningfulness of the cfDNA data is limited because only eight system exchanges were analyzed from one single patient. In addition, blood sampling for cfDNA detection was inhomogeneous before and after exchange. The first samples before exchange were collected in a time range of 1 to 7 days, and within 1 to 4 days after exchange. An extension of the sample collection—a daily blood collection—would be absolutely necessary to improve the validity of NET concentrations on ECMO. However, the additional daily blood sampling and plasma preparation requires high logistics, which in many cases can only be implemented to a limited extent in the case of critically ill patients on the ICU. Furthermore, the detection of cfDNA instead of specific NET marker (e.g., citH3, myeloperoxidase, neutrophil elastase), is an unspecific analysis marker that identified released DNA from all dead cells and tissue.[38]

Limitations: This is a prospective study. Sample size was very small and sample collection was incomplete due to high logistical effort. In addition, only one method was used for the identification of cfDNA in plasma samples. Measurement of cfDNA with a fluorescent dye is very unspecific, since cfDNA is also a result of cell necrosis and apoptosis. Identification of NETs with more specific methods (histones or MPO-bound DNA) are indispensable.

5 | CONCLUSIONS

cfDNA are present in blood from patients that required VV ECMO. The concentration remained unchanged during therapy, after a system exchange and after the end of therapy. Based on current data, the usefulness of NET formation in the form of cfDNA as a predictor of clotting events remains questionable. Additional studies are necessary to verify our findings of cfDNA with more specified NET-markers to be an indicator for the development of circuit-induced coagulation disorders.

AUTHOR CONTRIBUTIONS

Maximilian P. Lingel conducted experiments, analyzed data, drafted the manuscript. Moritz Haus contributed to data interpretation and revised the manuscript. Lukas Paschke collected and processed blood samples, collected data. Maik Foltan contributed to data interpretation, maintenance of the ECMO database. Matthias Lubnow, Michael Gruber and Lars Krenkel contributed to study design, interpretation of data, and critically revised the manuscript. Karla Lehle designed concept, supervised the study, and critically revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Respective data are included in the article/Supplementary Material, and further inquiries from this study can be directed to the corresponding author.

ETHICS STATEMENT

The study was approved by the Ethics Committee of the University of Regensburg (vote no. 16-101-0322). Informed consent was waived as all ECMO devices are approved for clinical use. No personalized data and only routine laboratory parameters were used.

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SUPPORTING INFORMATION

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